

**Data Evaluation Report on the Acute Toxicity of Ethylenethiourea to Fish (*Cyprinodon variegatus*)**


PMRA Submission Number N/A

EPA MRID Number 47441201

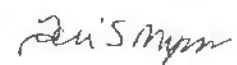
<b>Data Requirement:</b>	PMRA DATA CODE	{.....}
	EPA DP Barcode	D353731
	OECD Data Point	{.....}
	EPA MRID	47441201
	EPA Guideline	OPPTS 850.1075 (72-1)

<b>Test material:</b>	Ethylenethiourea; 2-imidazolidinethione	<b>Purity:</b> 100%
<b>Common name:</b>	Ethylenethiourea	
<b>Chemical name:</b>	IUPAC: Not Reported	
	CAS name: Not Reported	
	CAS No.: 96-45-7 (2-imidazolidinethione)	
	Synonyms: None Reported	

**Primary Reviewer:** John Marton  
Staff Scientist, Cambridge Environmental, Inc.

**Signature:**   
**Date:** 07/21/08

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**Date:** February 2015

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**Date:** 04/16/15

**Reference/Submission No.:** {.....}

<b>Company Code</b>	{.....}	[For PMRA]
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<b>Use Site Category:</b>	{.....}	[For PMRA]
<b>EPA PC Code</b>	600016	

**Date Evaluation Completed:** 11-02-2015

**CITATION:** Soucy, K. 2008. Ethylenethiourea- Acute Toxicity to Sheepshead Minnow (*Cyprinodon variegatus*) Under Static Conditions, Following OPPTS Draft Guideline 850.1075. Unpublished study performed by Springborn Smithers Laboratories, 790 Main St., Wareham, Massachusetts 02571-1037. Laboratory report number 13921.6105. Study submitted to EBDC/ETU Task Force, c/o McDermott, Will and Emery LLP, 600 13<sup>th</sup> St NW, Washington, DC 20005. Study submitted June 2, 2008.

**DISCLAIMER:** This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to fish. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

## **EXECUTIVE SUMMARY:**

In a 96-h acute toxicity study, sheepshead minnow (*Cyprinodon variegatus*) were exposed to ethylenethiourea at nominal concentrations of 0 (negative control), 63, 130, 250, 500 and 1000 mg ai/L under static conditions. The reviewer-calculated time-weighted average (TWA) concentrations were <6.3 (<LOQ; control), 54, 110, 228, 463 and 895 mg ai/L. The 96-h LC<sub>50</sub> was >895 mg ai/L. No mortality or sub-lethal effects were observed in the control or any of the ethylenethiourea treatment groups, yielding EC<sub>50</sub> and NOAEC values of >895 and 895 mg ai/L, respectively. Based on the results of this study, ethylenethiourea would be classified as practically non-toxic to sheepshead minnow in accordance with the classification system of the U.S. EPA.

This toxicity study is classified acceptable and satisfies the guideline requirement for an acute estuarine fish toxicity study.

### **Results Synopsis**

Test Organism Size/Age(mean weight or length): Weight- 0.28 (0.08-0.55) g; Length-24 (16-30) mm

Test Type (Flow-through, Static, Static Renewal): Static

LC<sub>50</sub>: >895 mg ai/L      95% C.I.: N/A

NOAEC: 895 mg ai/L      Probit Slope: N/A

EC<sub>50</sub>: >895 mg ai/L      95% C.I.: N/A

Endpoint(s) Affected: None

## **I. MATERIALS AND METHODS**

**GUIDELINE FOLLOWED:** This study was conducted following guidelines outlined in the U.S. Environmental Protection Agency's Ecological Effects Test Guideline (draft) OPPTS 850.1075- Fish, Acute Toxicity Test, Freshwater and Marine (U.S. EPA, 1996). The following deviations from OPPTS 850.1075 were noted:

1. The hardness of the dilution water was not reported. Furthermore, the results of a periodic screening analysis of the dilution water were not provided.
2. The measured pH values during the test (7.4-7.8) ranged slightly below the minimum recommended value (7.5-8.5).

These deviations do not impact the acceptability of the study.

**COMPLIANCE:** Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided. This study was conducted in compliance with all pertinent U.S. EPA Good Laboratory Practice regulations (40 CFR, Part 160) with the following exceptions: routine dilution water and food contaminant screening analyses for pesticides, PCBs and toxic metals were conducted at GeoLabs, Inc., Braintree, Massachusetts using standard U.S. EPA Procedures and are considered facility records. Since the analyses were conducted following standard validated methods, these exceptions had no impact on the study results.

### **A. MATERIALS:**

**1. Test material** Ethylenethiourea

**Description:** Solid

**Lot No./Batch No. :** 04816CH

**Purity:** 100%

**Stability of compound under test conditions:** Stable. Method validation was conducted by determining the recovery of ethylenethiourea from 0.1% trifluoroacetic acid in 20 ppt filtered seawater at fortified concentrations of 0, 0.300, 20.0 and 1000 mg/L. Individual recoveries ranged from 74.7 to 103% of nominal with a mean recovery of 93.4%.

*(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)*

**Storage conditions of test chemicals:** Stored in the dark at ambient temperature.

#### **Physicochemical properties of ethylenethiourea.**

Parameter	Values	Comments

Parameter	Values	Comments
Water solubility at 20°C	Not Reported	
Vapor pressure	Not Reported	
UV absorption	Not Reported	
pKa	Not Reported	
Kow	Not Reported	

## 2. Test organism:

**Species:** Sheepshead Minnow (*Cyprinodon variegatus*)  
**Age at test initiation:** Not Reported  
**Weight at study initiation:** 0.28 (0.08-0.55) g, N=30  
**Length at study initiation:** 24 (16-30) mm, N=30  
**Source:** Aquatic BioSystems, Inc., Fort Collins, Colorado.

## B. STUDY DESIGN:

### 1. Experimental Conditions

a. Range-finding study: A static 96-hour range-finding study was conducted by exposing 5 fish/level to nominal concentrations of 0 (negative control), 0.010, 0.10, 1.0, 10 and 100 mg ai/L. At test termination, no mortality or adverse effects were observed in the control or treatment levels. Based on the results and consultation with the Study Sponsor, nominal concentrations of 63, 130, 250, 500 and 1000 mg ai/L were chosen for the definitive exposure.

b. Definitive Study

**Table 1: Experimental Parameters**

Parameter	Details	Remarks
		Criteria
<u>Acclimation</u>		
Period:	At least 14 days	<i>The recommended acclimation period is a minimum of 14 days; OECD guideline recommends a minimum of 12 days. Pretest mortality should be &lt; 3% 48 h. prior to testing. OECD pretest mortality criteria: &gt;10% = rejection of entire batch; ≥ 5 and ≤ 10% = continued acclimation for 7 days; &lt;5% = acceptable.</i>
Conditions: (same as test or not)	Same as test	
Feeding:	The fish were fed Zeigler Brothers Prime Flakes, a dry commercial flaked food, <i>ad libitum</i> , daily except during the 48 hours prior to testing.	
Health: (any mortality observed)	No mortality was observed among the test fish population during the 7-day period prior to test initiation.	
Duration of the test	96 Hours	

Parameter	Details	Remarks
		Criteria
		<i>The recommended test duration is 96 hours.</i>
<u>Test condition</u>		
Static/flow-through	Static	<i>A reproducible supply of toxicant is recommended. Consistent flow rate is usually 5-10 vol/24 hours; meter systems should be calibrated before and after study and checked twice daily during test period.</i>
Type of dilution system - for flow-through method.	N/A	
Renewal rate for static renewal	N/A	
Aeration, if any	Gentle, oil-free aeration was initiated at the 72-hour observation interval to raise and maintain DO concentrations above 60% of saturation.	<i>Aeration is not recommended; OECD guideline recommends aeration. If aeration is necessary, test solutions must be analyzed periodically to verify exposure.</i>
<u>Test vessel</u>		
Material: (glass/stainless steel)	Glass	<i>Test vessel size is usually 19 L (5 gal) or 30 x 60 x 30 cm. Fill volume is usually 15-30 L of solution.</i>
Size:	39 x 20 x 25 cm	
Fill volume:	15 L	
Source of dilution water Quality:	Dilution water was prepared by filtering natural seawater collected from the Cape Cod Canal, Bourne, Massachusetts. The water was passed through a series of polypropylene core filters (20- and 5-micron). The seawater used during the study had a salinity range of 20 to 21‰ and a pH of 7.7.	<i>Recommended source of dilution water is soft, reconstituted water or water from a natural source. EPA does not recommend the use of dechlorinated tap water; however, its use may be supportable if the biological responses for the organisms and chemical analyses of residual chlorine meet conditions in the Agency's 850.1010 guidelines for dilution water (<a href="http://www.epa.gov/opptsfrs/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Draft/850.1010.pdf">http://www.epa.gov/opptsfrs/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Draft/850.1010.pdf</a>) Dilution water should be intensely aerated before the study. OECD permits dechlorinated tap water.</i>

Parameter	Details	Remarks
		Criteria
<u>Water parameters:</u> Hardness pH Dissolved oxygen Total Organic carbon Particulate Matter Metals Pesticides Chlorine Temperature {Salinity for marine or estuarine species} Intervals of water quality measurement	Not Reported 7.4-7.8 4.9-7.6 mg/L; >60% saturation 0.89 mg/L (February 2008) Not Reported Not Reported Not Reported Not Reported 21-23°C 20-22‰ Temperature, salinity, pH and DO were measured daily in each test vessel. Temperature was also monitored continuously in replicate B of nominal 1000 mg ai/L treatment level.  The study author reported that no pesticides, PCBs or toxic metals were detected at concentrations that are considered toxic.	Gentle, oil-free aeration was initiated at the 72-hour observation to maintain adequate DO saturation levels; however, prior to initiating the aeration, DO saturation levels did not drop below 60% (4.6-4.7 mg/L). <hr/> <u>Hardness:</u> EPA recommends 40 - 48 mg/L as CaCO <sub>3</sub> (OECD recommends 10 - 250 mg/L) <u>pH:</u> EPA recommends 7.2 - 7.6; 8.0-8.3 for marine-stenohaline fishes, 7.7-8.0 for estuarine-euryhaline fishes, monthly range < 0.8; (OECD recommends pH 6.0 - 8.5) <u>Dissolved Oxygen:</u> EPA recommends: Static: ≥ 60% during first 48 hrs and ≥ 40% during second 48 hrs; flow-through: ≥ 60%; (OECD guideline recommends at least 80% saturation value). <u>Temperature:</u> EPA recommends 12 EC for coldwater species, 17 or 22 EC for warmwater species, and 22 ± 1 EC for estuarine/marine organisms. (OECD recommends 21 - 25°C for bluegill and 13 - 17°C for rainbow trout). <u>Salinity:</u> EPA recommends 30-34‰ (parts per thousand) for marine, 10-17‰ for estuarine fish, weekly range < 6‰. Water quality should be measured at beginning of test and every 48 hours.
<u>Number of replicates/groups:</u> control: solvent control: treated ones:	2 N/A 2/level	Recommended number of replicates include a control and five treatment levels. Each concentration should be 60% of the next highest concentration; concentrations should be in a geometric series.
<u>Number of organisms per replicate /groups:</u> control: solvent control: treated ones:	10 N/A 10	Number of organisms per replicate should be ≥ 10/concentration; OECD guideline recommends at least 7 fish/concentration.

Parameter	Details	Remarks
		Criteria
Biomass loading rate	Not Reported; calculated by reviewer to be 0.19 g/L  0.28 g/fish x 10 fish/aquarium x aquarium/15L	<i>Recommended static conditions are #0.8 g/L at #17EC and #0.5 g/L at &gt; 17EC. Recommended flow-through conditions are #1 g/L/day. OECD recommends a maximum of 1 g fish/L for static and semi-static, while higher rates are recommended for flow-through.</i>
<u>Test concentrations:</u> nominal: measured:	0 (negative control), 63, 130, 250, 500 and 1000 mg ai/L  <6.3 (<LOQ; control), 54, 110, 228, 463 and 895 mg ai/L	The measured concentrations represent the reviewer-calculated TWA concentrations.  The mean-measured concentrations were 55, 110, 230, 470 and 990 mg ai/L
Solvent (type, percentage, if used)	N/A; a solvent was not used	<i>The solvent should not exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests; OECD recommends that the solvent not exceed 100 mg/L.</i>
Lighting	16L:8D; sudden transitions from light to dark were avoided. The test area was illuminated with fluorescent bulbs at an intensity range of 41-53 footcandles (440-570 lux) at the solutions' surface.	<i>The recommended photo period is 16 hours of light and 8 hours of dark with a 15-30 minute transition period. OECD recommends a photo period of 12 -16 hours.</i>
Feeding	Fish were not fed during the test.	<i>Fish should not feed during the study.</i>
<u>Recovery of chemical</u> Frequency of determination Level of quantization Level of detection	Samples were collected at 0, 48 and 96 hours 3.3-6.3 mg ai/L Not Reported	
Positive control {if used, indicate the chemical and concentrations }	N/A; a positive control was not used	
Other parameters, if any	None	

## 2. Observations:

**Table 2: Observations**

Parameter	Details	Remarks
		Criteria
Parameters measured including the sublethal effects/toxicity symptoms	-Mortality -Sub-Lethal Effects	Death was defined as the lack of movement by the exposed organisms (i.e., absence of gill movement and reaction to gentle prodding).
Observation intervals	0, 6, 24, 48, 72 and 96 hours	
		<i>Observation intervals should be a minimum of every 24 hours.</i>
Were raw data included?	No, summarized treatment level data were provided. These data were adequate for statistical analyses.	
Other observations, if any	All test solutions appeared clear and colorless without any undissolved test material.	

## II. RESULTS AND DISCUSSION:

### **A. MORTALITY:**

No mortalities were observed in the control or treatment groups at any point during the 96-hour exposure period, yielding NOAEC and LC<sub>50</sub> values of 900 and >900 mg ai/L, respectively, based on the mean-measured concentrations. The NOAEC and LC<sub>50</sub> values were 895 and >895 mg ai/L, respectively, based on the reviewer-calculated TWA concentrations.



**Table 3: Effect of Ethylenethiourea on Mortality of *Cyprinodon variegatus*.**

TWA and (Nominal) Concentrations mg ai/L	No. of Fish at Start of Study	Observation Period					
		Day 1		Day 3		Day 4	
		No Dead	% Mortality	No Dead	% Mortality	No Dead	% Mortality
Negative Control	20	0	0	0	0	0	0
54 (63)	20	0	0	0	0	0	0
110 (130)	20	0	0	0	0	0	0
228 (250)	20	0	0	0	0	0	0
463 (500)	20	0	0	0	0	0	0
895 (1000)	20	0	0	0	0	0	0
NOAEC <sup>1</sup>	895 mg ai/L						
LC <sub>50</sub> <sup>1</sup>	>895 mg ai/L						
Positive control, if used mortality: LC <sub>50</sub> :	N/A	N/A	N/A	N/A	N/A	N/A	N/A

<sup>1</sup> Values based on the reviewer-calculated TWA concentrations

N/A- Not Applicable

#### **B. NON-LETHAL TOXICITY ENDPOINTS:**

All surviving fish appeared normal and healthy throughout the 96-hour exposure period, yielding NOAEC and EC<sub>50</sub> values of 900 and >900 mg ai/L, respectively, based on the mean-measured concentrations. The NOAEC and EC<sub>50</sub> values were 895 and >895 mg ai/L, respectively, based on the reviewer-calculated TWA concentrations.

**Table 4: Sub-lethal Effect of Ethylenethiourea on *Cyprinodon variegatus*.**

TWA and (Nominal) Concentrations mg ai/L	Observation Period		
	Endpoints at Day 1	Endpoints at Day 3	Endpoints at Day 4
	% Affected	% Affected	% Affected
Negative Control	A.N.	A.N.	A.N.
54 (63)	A.N.	A.N.	A.N.
110 (130)	A.N.	A.N.	A.N.
228 (250)	A.N.	A.N.	A.N.
463 (500)	A.N.	A.N.	A.N.
895 (1000)	A.N.	A.N.	A.N.
NOAEC <sup>1</sup>	895 mg ai/L		
LOAEC <sup>1</sup>	>895 mg ai/L		
EC <sub>50</sub> <sup>1</sup>	>895 mg ai/L		
Positive control, if used % sublethal effect: EC <sub>50</sub> :	N/A	N/A	N/A

<sup>1</sup> Values based on the reviewer-calculated TWA concentrations

A.N.- All surviving fish appear normal and healthy

N/A- Not Applicable

### C. REPORTED STATISTICS:

During this study, no concentration tested resulted in  $\geq 50\%$  mortality, therefore, the LC<sub>50</sub> value was empirically estimated to be greater than the highest mean-measured concentration tested. The NOAEC value was determined using Kruskal-Wallis' Test. Data were first checked for normality using Chi-Square Test (Weber et al., 1989) and for homogeneity of variance using Bartlett's Test (Horning and Weber, 1985). These statistical determinations were performed using TOXSTAT® Version 3.5 (Gulley et al., 1996).

### D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The lack of mortality and sub-lethal effects precluded the statistical analysis of the data. All toxicity values were visually determined based on the reviewer-calculated TWA concentrations.

LC<sub>50</sub>: >895 mg ai/L      95% C.I.: N/A

EC<sub>50</sub>: >895 mg ai/L      95% C.I.: N/A

NOAEC: 895 mg ai/L

Probit Slope: N/A      95% C.I.: N/A

### E. STUDY DEFICIENCIES:

There were no study deficiencies.

## F. REVIEWER'S COMMENTS:

The reviewer's results were similar to those of the study author with the exception that the reviewer reported all toxicity values based on the TWA concentrations, while the study author reported all toxicity values based on the mean-measured concentrations. Therefore, the reviewer's results are reported in the Executive Summary and Conclusions sections of this DER.

The reviewer calculated the TWA concentrations using the following equation:

$$C_{TWA} = \frac{\left(\frac{C_1 + C_0}{2}\right)(t_1 - t_0) + \left(\frac{C_2 + C_1}{2}\right)(t_2 - t_1) + \left(\frac{C_{n-1} + C_2}{2}\right)(t_{n-1} - t_2) + \left(\frac{C_n + C_{n-1}}{2}\right)(t_n - t_{n-1})}{t_n}$$

where:

C TWA is the time-weighted average concentration,

C j is the concentration measured at time interval j (j = 0, 1, 2,...n)

t j is the number of hours (or days or weeks, units used just need to be consistent in the equation) of the test at time interval j

(e.g., t 0 = 0 hours (test initiation), t 1 =24 hours, t 2 =96 hours)

Analysis of the three QC samples yielded recoveries of 79.6-98.8% of nominal. The 79.6% recovery occurred at test initiation at the 1000 mg ai/L QC sample and this recovery was outside of the acceptable range (80-120% of nominal). However, the recovery at this level was 93.2% at 48 hours and 90.9% at 96 hours.

The initial measured concentrations, obtained from the samples collected from the test vessels, yielded recoveries of 84.6-92.0% of nominal. Recoveries at 48 hours were 92.9-100.0% of initially measured concentrations, while measured concentrations at test termination were 100.0-106.5% of initial measured concentrations. The 96-hour TWA concentrations yielded recoveries of 84.6-92.5% of nominal.

The parent compounds of the test material were [[1,2-ethanediyibis[carbamodithioato]](2-) manganese mixture with [[1,2-ethandiyibis[carbamodithioato]](2-)zinc.

The in-life portion of the definitive toxicity test was conducted from February 25 to February 29, 2008.

## G. CONCLUSIONS:

This study is scientifically sound and is thus acceptable for use in Agency risk assessments. The NOAEC, LC<sub>50</sub> and EC<sub>50</sub> values, based on a lack of mortality and sub-lethal effects, were 895, >895 and >895 mg ai/L, respectively.

LC <sub>50</sub> : >895 mg ai/L	95% C.I.: N/A
EC <sub>50</sub> : >895 mg ai/L	95% C.I.: N/A
NOAEC: 895 mg ai/L	
Probit Slope: N/A	95% C.I.: N/A

## III. REFERENCES:

ASTM. 2002. Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates and amphibians. Standard E729-96. American Society for Testing and Materials, 100 Barr Harbor Road, West Conshohocken, PA 19428.

- Gulley, D.D., Boelter, A.M. and Bergman, H.L. 1996 TOXSTAT® Release 3.5. University of Wyoming, Laramie, Wyoming.
- Horning, W.B. and C.I. Weber. 1985. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. EPA/600/4-85/014. Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio.
- U.S. EPA. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards; Final Rule (40 CFR, Part 160). U.S. Environmental Protection Agency, Washington, DC.
- U.S. EPA. 1996. Office of Prevention, Pesticides and Toxic Substances. Ecological Effects Guideline, OPPTS 850.1075. Fish Acute Toxicity Test, Freshwater and Marine. "Public Draft". EPA 712-C-96-118. April 1996. U.S. Environmental Protection Agency, Washington, DC.
- Weber, C.I., W.H. Peltier, T.J. Norberg-King, W.B. Horning II, F.A. Kessler, J.R. Menkedick, T.W. Heihsel, P.A. Lewis, D.J. Klemm, Q.H. Pickering, E.L. Robinson, J.M. Lazorchak, L.J. Wymer and R.W. Freyberg (eds.). 1989. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. 2<sup>nd</sup> ed. EPA/600/4/89/001. Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH.

Time-Weighted  
Averages  
(mg ai/L)

mg ai/L	0 Hr	% of Nominal	48 Hr	% of Initial	96 Hr	% of Initial	TWA (mg ai/L)	% of Nominal
Negative Control	<3.3	N/A	<6.3	N/A	<3.4	N/A	N/A	N/A
63	56	88.9	52	92.9	57	101.8	54	86.1
130	110	84.6	110	100.0	110	100.0	110	84.6
250	230	92.0	220	95.7	240	104.3	228	91.0
500	460	92.0	450	97.8	490	106.5	463	92.5
1000	900	90.0	870	96.7	940	104.4	895	89.5